SANITATION OF SOIL BY MICRO-ORGANISMS

N. A. KRASILNIKOV

Institute of Microbiology, Academy of Sciences, Moscow, U.S.S.R.

INTRODUCTION

Many crop plants and the soils that they grow in may be infested with harmful forms of micro-organisms, e.g. phytopathogenic bacteria and fungi, nematodes, viruses and other living organisms. Such contamination of soil by these microbial 'weeds' is often due to the violation of agrotechnical and agrobiological regimes or to the occurrence of unsuitable climatic conditions. In certain cases, the contamination of soil by pathogenic microbes may be so great, that these soils become useless from a practical point of view. Plant yields may be considerably reduced. For example, in England it has been estimated that up to £140,000,000 worth of crops are lost every year. Prasada (1965) reported that in India the loss of wheat alone was more than one million tons per annum. Similar losses are experienced in most other countries. According to the data supplied by FAO, the losses of corn due to pathogens may reach 33 million tons per annum. This is sufficient to feed 150 million people for one year. Further, it has been stated (International Congress for Plant Protection, Hamburg, 1957), that the losses due to pathogenic microbes and insects are one fifth of the total harvest for wheat, one sixth for beans and potatoes, and one half for apples. When a monoculture system is used, mass infection of plants is often observed in fields sown with perennial grasses, corn, cotton and other species of plants.

RESULTS

In our country we experience great losses because of pathogenic microbes, e.g. in the cotton harvests in Central Asia. For the last 30 years in Usbekistan, the cotton wilt disease has been increasing year by year (Table 1). According to our observations, contamination of sierozem soils with fungi in the cotton fields in Usbekistan and Tadjikistan increased noticeably with each consecutive sowing of this plant. Microbial analyses of these soils showed a gradual increase in the count of the fungus causing wilt, *Fusarium vasinfectum* (Table 2).

TABLE 1

The annual increase of wilt-disease of cotton, expressed as the percentage of infected plants

Year	1937	1938	1959	1960	1961	1962	1963
Infected plants	1–3	1–2	10	22	30	46	42

TABLE 2

Degree of infection of the soil with the fungus, Fusarium vasinfectum *in monocultured cotton-fields*

Sowing	Control soil		Cotton sown soil	
	Total fungi	*F. vasinfectum*	Total fungi	*F. vasinfectum*
1st and 2nd	20–40	0–0·01	20–40	0–0·02
3rd	15–30	0–0·05	20–40	1·5–5·0
5th	40–60	0·1–0·2	60–80	20–40
6th	30–40	0–0	100–200	30–40
7th	50–60	0·01–0·05	100–200	50–60
8th	50–70	0–0·2	180–200	50–80

Numbers of fungi are expressed in thousands/g. of soil.

N.B. The control soil was from the same field but from an area in which crop rotation was being practised.

It is apparent from this data that severe contamination of soil occurred after the 5th consecutive sowing of cotton in the 5th year. Contamination with the fungus *Fusarium vasinfectum* reached a maximum in the 8th year, when the fungal count was 8×10^3 propagules per g. of soil. We found a positive correlation between the increases in the number of pathogenic fungi in the soil and the percentages of cotton plants infected with wilt disease.

Similar results were obtained from studies on the infection of flax, clover and other plants grown in monoculture. Soils cultivated with these plants become more and more saturated with phytopathogens year by year. Table 3 shows the results of our analyses of soils over several years in experimental plots of the Timiryazeva Academy of Agriculture near Moscow, which have been established for many years with a flax crop.

The fungus *Fusarium lini* developed abundantly in soils repeatedly

TABLE 3

Infestation of flax plants and soil with fungi

Year of sowing	Flax-monoculture—soil		Plants sown	Normal soil	
	F. lini thousands/g.	Infection of flax (%)		*F. lini* in thousands/g.	Infection of plant (%)
1st year Flax	0–0·1	0–0·02	Flax	0	0
2nd ,, ,,	0·1–0·9	0–0·05	Oats	0	0
3rd ,, ,,	2–3·5	3–5·5	Clover	0	0
4th ,, ,,	5–10	10–20	Clover	0	0
5th ,, ,,	20–50	30–40	Clover	0·5	0·1
6th ,, ,,	60–80	50–60	Wheat	0	0

cultivated with flax over a long period, reaching a count of 50 thousand to 100 thousand propagules per g. of soil. This same soil, when cultivated with other plants, did not contain this pathogen or else it was observed only in rare cases.

It can be seen that the cotton plant grown in a sierozem, and the flax grown in a podsol, favoured the development and accumulation of pathogenic fungi of the genus *Fusarium* in the soil.

Soil is not only contaminated by wilt fungi. Appreciable contamination of soil with phytopathogenic fungi of the genera *Helminthosporium*, *Rhizoctonia*, *Verticillium*, etc. can be observed. Contamination of soil by bacteria pathogenic to man often occurs. Soils of towns, industrial centres, and other densely populated areas are often contaminated with bacteria of the coliform group. Certain fields may occur which are contaminated by the causative agent of Siberian ulcer (*Bacillus anthracis*); the accumulation of *Mycobacterium tuberculosis* in soil near hospitals and sanatoriums has been noted.

In all cases of contamination of soil by harmful micro-organisms, sanitary measures are essential to remove these pathogenic and phytopathogenic forms. In practice, methods for soil improvement do not always give the desired results. Soils may be treated with various chemicals; over the last few years pesticides, herbicides and fungicides have been used widely, together with methods involving complete or partial soil sterilization. It should be remembered, however, that it is only possible to carry out soil sterilization with small volumes of soil in greenhouses and seed beds or in small garden plots. Complete sterilization is never achieved and is not necessarily desirable since it destroys certain useful micro-organisms.

Application of pesticides often produces highly undesirable results. Wide-spread application and long-term use of pesticides or biocides leads not only to the poisoning of soils and the destruction of both harmful and useful organisms, but also to the poisoning of plants, wild animals and birds. They are also responsible for the poisoning of reservoirs and all the living creatures in them. Uncontrolled use of pesticides does more harm than good.

Moreover, pesticides often affect not only the pathogenic forms but also their antagonists. As a consequence, the soil does not improve: on the contrary, it becomes contaminated. The phytopathogenic forms, not finding competitors or inhibitors, begin to multiply and occur in the soil in much higher numbers than before the treatment.

As an example, I shall quote our data concerning analyses of soils treated with separate chemicals. Samples of sierozem soil from Tadjikistan and podsol soil from the Experimental Station near Moscow were inoculated with *F. vasinfectum* and *F. lini* respectively and then treated with simazine (5 mg./kg.), followed by a preparation of 2-4D (10 mg./kg.) or 3-chloracetate (300 mg./kg.). After a period of 5–10 days, the numbers of mycolytic bacteria in the soil were counted. The results are shown in Table 4.

TABLE 4

The influence of chemical poisons on the process of soil contamination with pathogens. (bacteria in thousands and fungi in units/g.)

Chemicals	Dose of Chemicals	Sierozem soil		Podsol soil	
		Mycolytic Bacteria/g. soil	Fusarium vasinfectum/g. soil	Mycolytic Bacteria/g. soil	Fusarium-lini/g. soil
Simazine	5	500	0–100	0–0·1	50000
2-4D	10	1500	0–0	50000	0–0
3-chloracetate	300	0	10000	0	80000
Control soil		100000	0–0·1	150000	0–0

The results show that, under the experimental conditions, simazine inhibited the mycolytic bacteria which lyse the mycelium of *Fusarium lini*, as a consequence of which, the latter multiplied markedly in the podzol. 3-chloracetate completely inhibited the mycolytic bacteria, whereas 2-4D did not inhibit these bacteria.

Negative results from the use of chemical poisons has encouraged specialists to concentrate their attention on biological factors associated with the control of organisms harmful to plants. According to our observations microbial antagonism is a particularly effective means for the general improvement of soil and protection of plants. The phenomenon of antagonism is widespread in the microbial world, especially in the soil. Antagonists can accumulate in soil in millions or hundreds of millions per gram (Krasilnikov, 1958; Agre, 1964; Hussein, 1964; Tzi-Shen, 1961).

Antagonists nearly always exist in soil which are able to inhibit actively the growth of pathogenic bacteria and fungi. In cases where the antagonists develop abundantly in the soil and accumulate there in sufficiently large quantities, phytopathogenic forms do not develop. Because of this, the principle of soil improvement using micro-organisms has been established, i.e. the enrichment of soil with antagonists and the utilization of certain microbes for the control of other microbes. Is this useful in practical agriculture? I think so. There are experiments, although not many, which show that soils, artificially enriched with antagonists, contain no pathogenic bacteria. Such experiments were carried out by Bogopolski (1948, 1950) with the coliform organisms and by Afrikian (1951) with *Azotobacter* and the spore-forming bacterium *Bacillus cereus mycoides*. Krasilnikov (1944) observed the inhibition of staphylococci in soil enriched with antagonists. Arkhipov (1954) noted the death of *Bacillus anthracis* in the soil when bacterial antagonists had accumulated. Lastly, Gubkin (1963) observed the death of *Salmonella*.

The inhibition of phytopathogenic fungi in the soil by antagonistic microbes has been observed by many workers. Fungi such as *Helminthosporium*, *Fusarium*, *Phytophthora*, *Verticillium*, *Rhizoctonia*, *Phymatotrichum* and *Alternaria* can be inhibited and eliminated from the soil by bacteria and other antagonistic micro-organisms (Krasilnikov, 1958; Stover, 1963; Baker, 1963; Alexander, 1964; Mitchell, 1963).

With regard to the question of whether it is possible to artificially enrich the soil with antagonists, the answer is decidedly positive. Two methods of enrichment of soils are possible:

1. The accumulation of antagonists with the help of plants.
2. The inoculation of appropriate antagonists into the soil.

Firstly plants are selected for crop rotation which encourage the development and accumulation of the antagonists. It is well known that plants differ in their ability to influence micro-organisms in the soil. Some plants

favour the development of one group of microbial species whilst others attract and increase the growth of quite different organisms by means of root secretions. Certain plants favour the accumulation of phytopathogenic microbes, whereas others favour the growth of the antagonists. For example, in the sierozem soils of Central Asia under perennial cotton, the soil is enriched with the wilt-causing fungi, *Verticillium dahliae* and *Fusarium vasinfectum*. Lucerne, however, encourages the growth of mycolytic bacteria and other microbial antagonists, which inhibit the growth of these fungi. Thus, before the sowing of lucerne after a 7 year cotton culture, there were 10–100 cells of mycolytic bacteria per g., but at the end of the first year with lucerne (sown after cotton), the number had increased to 100–500 thousand/g. and after 2–3 years of lucerne cultivation to 10–100 million/g. Table 5.

TABLE 5

Distribution of mycolytic bacteria in soil under cotton and lucerne in crop rotation. (millions/g. soil)

Region	Antagonists of *V. dahliae*				Antagonists of *F. vasinfectum*			
	Lucerne			Cotton	Lucerne			Cotton
	1st year	2nd year	3rd year	7th year	1st year	2nd year	3rd year	7th year
Usbekistan Experimental Station STAZR	0·1–0·5	1–15	10–50	0–0·01	0–0·2	15–30	20–50	0·01
Field near Tashkent	0·5–0·8	1·5–10	15–40	0·01	0·5	5–40	10–100	0·05
Tadjikistan Experimental Station, Vaksh	0·3–1·0	10–40	30–100	0·005	1·5	10–60	50–100	0·0
Field near Kurgan Tube	0·3–0·6	5–25	20–60	0·002	0·5	60	80–150	0·01

These data show that the degree of development of mycolytic bacteria is inversely proportional to the degree of development of the fungi that cause the infection. Cotton farmers have known for a long time that cotton wilt sharply declines after the inclusion of lucerne in crop rota-

tion schemes for 2–3 years; this is a practical method of improving the fields.

In certain parts of the U.S.A., *Arachis* has been introduced into the crop rotation with cotton for soil sanitation purposes. *Arachis*, during development, inhibits and reduces the percentage of infected plants. Presumably *Arachis* favours the development of antagonists active against the wilt-causing fungi.

It is also possible to carry out soil improvement by means of suitable fertilizer treatment, mineral or organic. Voronkevich (1958) treated soil with lime, a mixture of lime and peat and manure. She reported a marked decrease in the soft rot bacterium, *Erwinia carotovora*, with a simultaneous increase of antagonistic actinomycetes. Similar observations were made by workers at the Rothamsted Experimental Station in England. According to their data, the introduction of chitin into the soil caused enrichment of the antagonists of the wilt disease fungus of peas, *Fusarium albo-atrum* (Rothamsted Experimental Station Report, 1963). This was also observed by Mitchell (1963) and Alexander (1964). They showed that the introduction of chitin or chitin-containing substances (mycelia of fungi, etc.) into the soil lead to the increased development of certain bacteria which produce the enzymes chitinase and laminarinase and consequently lyse the cell walls of phytopathogenic fungi, e.g. *Fusarium*, which contain chitin and laminarin. Sequeira (1962) added sugar and sugar cane to the soil. Both caused increased development of bacteria which inhibited the growth of fungi by causing lysis of their mycelia. Baker (1963) and Stover (1963) showed that application of organic matter to the soil increased the numbers of bacteria and actinomycetes which were antagonistic towards phytopathogenic fungi. Wierbicki (1961) reported the improvement of soil irrigated with sewage, the organic matter of the latter eliminating pathogenic bacteria from the soil. In such fields grazing cattle were not exposed to intestinal infection.

Our investigations show that specially composted microbial preparations of antagonists, bacteria or actinomycetes, inhibited the development of the phytopathogenic microflora in the soil. Composts can be prepared from peat mixed with soil. These substrates are inoculated with special isolates of antagonistic actinomycetes or bacteria, and incubated for 10–15 days. During this time the bacteria or actinomycetes germinate within the compost. After adding such a preparation to the soil the antagonists survive and develop to a greater degree. If the antagonists do not survive in the soil they will not produce the expected effects and such preparations will be worthless. Strains must be used

which have the ability not only to inhibit the given disease-producing organism but also to survive and develop abundantly in the soil, particularly in the rhizosphere.

Preliminary experiments were carried out in pots and in special test-plots at experimental stations in Usbekistan and Tadjikistan; final experiments were carried out in commercial fields. In all cases where a good compost was established the results were positive; the infection with cotton wilt disease decreased by 40 to 70 per cent (Table 6).

TABLE 6

The influence of compost preparations of actinomycete antagonists on the incidence of cotton wilt

Treatment		Infected plants (%)		Yield in g. × 10^2/hectare
		Cotyledon stage	Fruiting stage	
Control		74·4	19·1	16·4
Compost treated with antagonistic strain number	114	8·1	0·01	34·8
	160	10·7	0·05	35·1
	117	10·2	0·4	25·6
	66	18·4	2·0	18·2

The best effect was obtained with composts prepared from strains of actinomycetes No. 114 and No. 160. The percentage infection was minimal, and the harvest maximal (34·8-35·1 × 10^2 g. per hectare). Microbial analyses of the soil with these treatments in the experiment showed that the organism causing wilt (*Fusarium vasinfectum*) had sharply declined in number. The count was 0 to 100 propagules/g. compared with the control soil where the count was 100 thousand to 500 thousand propagules/g. Such experiments were conducted by Kublanovska, Korenyako, Kuzina and Rasnitsina (Krasilnikov, 1958) and in recent years by Khodjibayeva (1965). Kublanovska tested composts with actinomycetes and these had just as effective results as the best fungicide at that time, granazan, in the control of *Verticillium* wilt of cotton. Rasnitsina used composts with mycolytic bacteria for the control of the wilt of pine seedlings in badly infected fields near Moscow. In control plots the loss of pine seedlings was over 80 per cent compared with the treated plots where the losses were from 10–15 per cent (Table 7). In our opinion the above data is sufficiently convincing to show the value of microbial methods for the improvement of soil.

TABLE 7

The inhibition of wilt of pine seedlings by bacterial composts (calculated at the end of the second year of growth)

Treatment	Count of *Fusarium* spp. per g. of soil	Survival of Plants (%)	Plant height (cm.)
Control	150,000	3	7·0–8·0
Addition of mycolytic bacteria (strain 30)	200	86	10·2
,, ,, (strain 18)	0·4	88	12·1
,, ,, (strain 23)	0·1	90	12·8
Addition of actinomycetes	0–100	85	10·8

Antagonists develop not only under experimental conditions in the laboratory, but also under natural conditions, directly in the soil. This is a fact beyond doubt. It is only necessary to use the antagonists skilfully to control pathogenic forms and remove them from soil. Further research is necessary, however, particularly in relation to the commercial value of the method under field conditions.

CONCLUSIONS

Soil infestation with pathogenic fungi and bacteria has often been observed. In recent years, in many areas, noticeable increases in the degree of infestation of soil with harmful micro-organisms have been reported. As a result, mass infection of plants takes place and very poor harvests result. Infestation of soil with pathogens is often seen after monoculture of a crop has occurred in the field over a number of years. After 7–8 years of cotton cultivation, the quantity of *Fusarium vasinfectum* and *Verticillium dahliae* sharply increase and reach a maximum count of up to 80 thousand propagules per g. Their antagonists, bacteria or actinonycetes, sharply decrease or completely disappear. Similar changes can be observed in soil under monocultured flax or pine.

It is possible to remove pathogenic fungi and bacteria from soil with the help of microbial antagonists. It has been established that with the abundant development of antagonists in the soil, the phytopathogens sharply decrease and in some cases are completely destroyed. Of all the soil micro-organisms, the most effective antagonists of the phytopathogenic fungi (*Fusarium* and *Verticillium*) are the mycolytic, non-sporeforming bacteria and actinomycetes. Cultures of bacteria and

actinomycetes have been isolated which actively inhibit the development of pathogenic microbes in the soil. Several methods for the practical use of these antagonists have been worked out. These methods are based upon two principles:

(*a*) the establishment of a crop rotation allowing the accumulation of antagonists in the soil,
(*b*) the production of special composts containing antagonists.

The use of these methods improves infected soil by removing pathogenic microbes.

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DISCUSSION

BACTERIA IN THE ROOT REGION OF PLANTS

Chairman: D. Parkinson, The University of Waterloo

Prof. D. Parkinson. Dr. Fåhraeus described the very intimate association of *Rhizobium* spp. with legume roots. The morphological effects of micro-organisms on living roots are only just beginning to be understood and the peculiar morphological effect of the rhizobia is surely extremely interesting. In this connection, the role of electron microscopy in studying microbial colonization of roots is becoming increasingly important. Many of you will probably remember the photographs, published in the California Agricultural Bulletin by Jenny and his colleagues, of the bacteria in the mucigel on roots.

Dr. Macura emphasized the role of the root exudates in the qualitative and quantitative expression of the rhizosphere effect. I think that he raised an important question with regard to rhizosphere ecology when he emphasized that the rhizosphere is a group of microhabitats, not a uniform microhabitat, that the situation in the root tip region may be ecologically very different from that in the crown of the root system. Also, he touched on the problems of foliar application of chemicals. This technique is a very powerful experimental tool for studying rhizosphere colonization and metabolism in the root region.

Professor Krasilnikov raised a number of very important questions with regard to biological control; he presented very clear-cut data on this problem. The effects of continuous crop treatment in building up pathogenic populations in soil is well known and presumably is, at least in part, an expression of the rhizosphere effect.

I would like to open the discussion with a question to Professor Krasilnikov. What methods did you use for counting mycolytic bacteria in the soils with which you were dealing?

Prof. N. A. Krasilnikov. I used a modification of the extinction dilution method. I grew cultures of *Fusarium vasinfectum* and *Fusarium lini* in tubes. Then I took the sample of soil to be analysed and dispersed it in water on a shaker. I prepared a series of dilutions from it and inoculated the different dilutions into the cultures of *Fusarium*. The mycelium was destroyed by those samples which contained mycolytic bacteria. By counting the tubes in which mycolysis occurred, I could calculate the most probable numbers of bacteria present.